An Easy Way of Demonstrating the Nuclei of Nerve Fibres.

By

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While attending the histology class this summer, I, in common with my neighbours, found great difficulty in rendering visible the nuclei of teased, fresh nerve fibres. Using gentian violet as recommended in Sir Edward Schäfer's 'Essentials of Histology,' the nerve fibres were almost uniformly stained, and the nuclei did not stand out convincingly. I tried other stains with equally disappointing results, and at last, in desperation, I used a mixture of nearly all the stains on the table (Ehrlich's hæmatoxylin, methylene blue, and alcoholic solution of eosin) and thus obtained a preparation in which blue nuclei stood out prominently on a reddish background. The preparation was shown to Dr. de Souza and Prof. Halliburton, who suggested to me that I should proceed to investigate the matter and ascertain what was the cause of success in this "blunderbuss" experiment.

Without going into all the details of the numerous preparations I made subsequently, I may state at the outset that the principal factor is the alcohol, in which, in my first successful experiment, the eosin had been dissolved. Aqueous solutions of eosin are quite as ineffective as the other stains. Fresh nerve fibres (and especially their nuclei), teased on the slide in the usual way, stain with great difficulty. But the nuclei stain readily with practically any dye (methylene blue, hæmatoxylin, picro-carmine, gentian violet) after preliminary treatment with alcohol. Or if the dye is added first and the nuclei remain unstained, the stain in the nuclei becomes evident on subsequent addition of alcohol to the preparation.

The difficulty of staining these nuclei in fresh preparations appears to have been noticed by others. Thus in Foster and Langley's 'Practical Physiology and Histology' (7th edition, p. 125) I find this statement: "The nuclei of the sheath may be stained by placing a piece of nerve after brief treatment with osmic acid in picro-carmine or hæmatoxylin for an hour." In Stirling's 'Practical Histology' (2nd edition, p. 206) the directions for staining the nuclei include the statement that after osmic acid picro-carmine may be used, but it is best to leave them for several days in the stain.

In consequence of these statements I made numerous preparations in order to see whether preliminary treatment with osmic acid will take the place of the alcohol, but with very indifferent results. Hæmatoxylin after osmic acid gave a brownish appearance to the whole nerve fibre, but the nuclei did not stand out; and in the case of gentian violet after osmic acid the nuclei were apparent because they were stained less darkly than the rest of the fibre. Picro-carmine after osmic acid did stain the nuclei red, but this took a considerable time, and the preparations were not nearly so good as after treatment with alcohol. In A. B. Lee's 'Microtomists' Vade-mecum' (7th edition, p. 136) I find the following, which seems germane to the present question: "Living tissue elements in general do not stain at all, but resist the action of colouring reagents till they are killed by them. . . . Objects which have been passed through alcohol generally stain better than those which have only been in watery fluids. But long preservation of tissues in alcohol is generally unfavourable to staining."

It is well known that the nuclei of nerve fibres are usually quite well stained in sections, and in this case alcohol is usually employed in the stages of preservation or embedding.

I can, however, confirm Lee's statement that long preservation in alcohol is not beneficial to the staining of nerve nuclei; after a nerve has been kept for some days in alcohol it is almost as difficult to stain its nuclei as when it is in the fresh condition.

The following is the method I would recommend for general routine work when a rapid result is wanted. The fresh nerve is teased on a dry slide in the usual way, the preparation being kept moist with the breath. A drop of absolute alcohol is added and then a drop of Ehrlich's hæmatoxylin, followed by a drop of methylene blue. Either dye may be used alone, but the nuclei are most deeply stained when both are employed. An alcoholic solution of eosin may be substituted for the absolute alcohol; the alcohol here is the essential reagent, but the eosin provides a red counter-stain. The preparation is then washed, cleared, and mounted in the usual way, and the whole operation is completed within a few minutes.